AMENDMENTS TO THE SPECIFICATION

Please replace the last paragraph on page 6 of the specification with the following rewritten paragraph:

Figure 2. The rP-8/4x and rP-8/4x-MD Ribozymes Base Pairing with Various Substrates. 2A) The *P. carinii* ribozyme, rP-8/4x (uppercase lettering, SEQ ID NO:9), binding to the 36-mer substrate (lowercase lettering with a gray background, SEQ ID NO:10). In the trans-excision-splicing reaction, the bridging region (white lettering) is excised and the 5' and 3' regions of the substrate (black lettering) are subsequently spliced together. Note that P1, P9.0, and P10 are helices that result from the recognition elements RE1, RE2, and RE3 base pairing with the substrate. The large bold arrows indicate the sites of catalysis for the first (left) and second (right) step of the trans-excision-splicing reaction. The 5' uridine and 3' guanosine are circled. The ribozyme bases are numbered according to that for the *P. carinii* intron (Testa, S. M., Haidaris, C. G., Gigliotti, F., & Turner, D. H. (1997) Biochemistry 36, 15303-15314.13, incorporated herein in its entirety). 2B). Simplified diagrams of various substrates base pairing with various ribozymes. Only the recognition element sequences are shown for the ribozymes. The dashed line indicates a normal phosphodiester bond between the adjoining sequences. i) The 12-mer substrate (SEQ ID NO:7) binding to rP-8/4x (SEQ ID NO:11). ii) The 10-mer substrate (SEQ ID NO:8) binding to rP-8/4x (SEQ ID NO:11). iii) The 38-mer Myotonic Dystrophy substrate (SEQ ID NO:12) binding to the rP8/4x-MD Myotonic Dystrophy ribozyme (SEQ ID NO:13).

Please replace the first paragraph on page 7 of the specification with the following rewritten paragraph:

Figure 3. The Trans-Excision-Splicing Reaction using the *P. carinii* system. The Trans-Excision-Splicing (TES) Reaction Using the *P. carinii* System. A) Polyacrylamide gel showing substrates and products of the TES reaction using 166 nM rP-8/4x ribozyme and 1.33 nM substrate at 7 mM $MgCl_2$ (36-mer) and 10 mM $MgCl_2$ (10-mer and 12-mer) at 44 °C. The reaction using the 36-mer substrate is diagrammed on the left (SEQ ID NO: 10, SEQ ID NO:14, and SEQ ID NO:15). The regions of the substrate that bind to the ribozyme's recognition elements (labeled RE1 (SEQ ID NO:15), RE2, and RE3) are underlined. All reactions in the presence (+) and absence (-) of the rP-8/4x ribozyme were subjected to the same incubation

conditions. TES reactions were conducted using a 36-mer substrate (to give a 16-mer product), a 12-mer substrate (9-mer product), and a 10-mer substrate (9-mer product). See Figure 2 for the sequence of these substrates. The 6-mer lane shows a synthetic control for the 5' cleavage products, the 16-mer lane shows a synthetic control for the 16-mer TES product, and the $^{-}$OH lane shows an alkaline digest of the 36-mer starting material. B). Graphs of TES reactions using the 36-mer substrate. All reactions were run as above except for the changing variable. The TES product is represented by filled circles and the 5' cleavage product by open circles. C). Graphs of TES reactions using the 10-mer substrate. All reactions were run as above except for the changing variable. The TES product is represented by filled triangles and the 5' cleavage product by open triangles. Each graph shows the average of two independent assays.

Please replace the first full paragraph on page 8 of the specification with the following rewritten paragraph:

Figure 5. Competition TES Reactions. Polyacrylamide gel showing substrates and products of TES reactions using 166 nM rP-8/4x, 7 mM $MgCl_2$, and 44 °C. 'Radiolabeled substrate' refers to the length of the radiolabeled substrate at 1.33 nM final concentration and 'cold substrate' refers to the length of the non-radiolabeled substrate. The final concentrations of the cold substrates are 1.33 nM for the 36-mer, and 66.5 nM (50X) or 665 nM (500x) for the 7-mer, which is r(GUGCUCU) (SEQ ID NO:16). The two substrates for each reaction were added simultaneously. Lanes designated in the first box are length controls, the second box shows the 5' exon competition assay, the third box shows an alkaline digest of the 36-mer starting material, and the fourth box shows the 3' exon competition assay.

Please replace the last paragraph on page 8 and continuing on to page 9 of the specification with the following rewritten paragraph:

Figure 6. The TES Reaction Using the DMPK Model System.. A) Polyacrylamide gel showing substrates and products of the TES reaction using 166 nM rP-8/4x-MD ribozyme, 1.33 nM substrate, 13 mM $MgCl_2$, and 44 °C. The reaction using the 38-mer DMPK mimic is diagrammed on the left (SEQ ID NO: 12, SEQ ID NO:17 and SEQ ID NO:18). The regions of the substrate that bind to the ribozyme's recognition elements are labeled RE1 (SEQ ID NO:18), RE2, and RE3. All reactions in the presence (+) and absence (-) of the rP-8/4x-MD ribozyme

were subjected to the same incubation conditions. TES reactions were conducted using the 38-mer substrate (to give a 10-mer product). The 6-mer lane shows a synthetic control for the 5' cleavage products, the 10-mer lane shows a synthetic control for the 10-mer TES product, and the $^{-}$OH lane shows an alkaline digest of the 38-mer starting material. The lanes labeled 36 are TES reactions using the rP-8/4x-MD ribozyme with the 36-mer *P. carinii* substrate (at 13 mM $MgCl_2$), and the lane labeled 38 (lane o) is a reaction using the rP-8/4x ribozyme with the 38-mer DMPK mimic (at 7 mM $MgCl_2$). In these cases, no reaction occurs. B). Graphs of TES reactions using the 38-mer substrate and rP-8/4x-MD. All reactions were run as above except for the changing variable. The TES product is represented by filled squares and the 5' cleavage product by open squares.

Please replace the first full paragraph on page 9 of the specification with the following rewritten paragraph:

Figure 7. TES reactions using two different *Tetrahymena* ribozymes. Figure 7A is a polyacrylamide gel showing substrates, intermediates, and products of the TES reaction. The reaction was carried out for 90 minutes using 166 nM ribozyme and 1.33 nM radiolabeled 13-mer substrate at either 0 mM or 10 mM $MgCl_2$ at 44 degrees C, either in the presence (+) or absence (-) of 330 nM GMP. Figure B is a diagram of the TES reaction (SEQ ID NO:19 and SEQ ID NO:20). The excised G of SEQ ID NO:19 is in bold lettering. Figure 7C shows two graphs of TES reactions using the 13-mer substrate and the A-L-21 Sca ribozyme (*Tetrahymena*). The TES product is represented by the filled circles and the 5' intermediate (CUCUCU) (SEQ ID NO:21) is represented by open circles. All reactions were run as above except for the changing variable. Each curve represents the average of two independent assays. Standard deviation for all points was less than 10%. For clarity the graphs use different scales.

Please replace the last paragraph on page 20 and continuing on to page 21 of the specification with the following rewritten paragraph:

Plasmid construction and synthesis. The *P. carinii* ribozyme plasmid precursor, P-8/4x, was generated as previously described (Testa, S. M., Haidaris, C. G., Gigliotti, F., & Turner, D. H. (1997) *Biochemistry* **36**, 15303-15314). The Myotonic Dystrophy-specific ribozyme plasmid precursor, P-8/4x-MD, was derived from the P-8/4x plasmid by site-directed

mutagenesis. Briefly, three successive rounds of mutagenesis were performed to modify each of the three recognition elements using the following pairs of mutagenic primers (underlined bases represent altered recognition elements as compared to P-8/4x): $^{5'}$CACGCCGCTTTCGGGAACCTCTATAGTGAGTCG$^{3'}$ (SEQ ID NO:~~NO.~~ 1) and $^{5'}$CGACTCACTATAGAGGTTCCCGAAAGCGGCGTG$^{3'}$ (SEQ ID NO:~~NO.~~ 2) for RE1 formation, $^{5'}$GGTATAGTCTTGCCTCTTTCGAAAG$^{3'}$ (SEQ ID NO:~~NO.~~ 3) and $^{5'}$CTTTCGAAAGAGGCAAGACTATACC$^{3'}$ for RE2 (SEQ ID NO:~~NO.~~ 4) formation, and then $^{5'}$CGACTCACTATAGGTGTTCCCGAAAGCGGC$^{3'}$ (SEQ ID NO:~~NO.~~ 5) and $^{5'}$GCCGCTTTCGGGAACACCTATAGTGAGTCG$^{3'}$ (SEQ ID NO:~~NO.~~ 6) for RE3 formation. Each set of primers (15 pmol each primer) was used in an amplification reaction comprising 25 ng parental plasmid, 2.5 units Pfu DNA polymerase (Stratagene; La Jolla, CA), and 0.5 μM dNTPs in a buffer comprising 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 2 mM $MgSO_4$, 0.1% Triton X-100, and 0.1 mg/mL BSA (final volume 50 uL). After an initial denaturation for 30 seconds at 95 °C, the mixture was subjected to 15 cycles of 95 °C for 30 seconds, 50 °C for two minutes, and 68 °C for six minutes. Parental plasmid was then digested with 20 units *Dpn* I (Gibco BRL; Rockville, MD) in 5.7 μL of the manufacturer's supplied buffer for 2 hours at 37 °C. 3 μL of this mixture was then used to transform *E. coli* DH5α competent cells (Gibco BRL). The vectors were purified using a QIAprep Spin Miniprep Kit (QIAGEN; Valencia, CA). The resultant final plasmid, P-8/4x-MD, was sequenced for confirmation (ACGT, Inc; Northbrook, IL). The plasmids were linearized with Xba I, phenol/chloroform extracted, and ethanol precipitated in preparation for run-off transcription.

Please replace the last paragraph on page 23 of the specification with the following rewritten paragraph:

To determine if the 3' product of the first reaction step (5' cleavage) (Figure 1B) is dissociating and then rebinding the same (or different) ribozyme before the second reaction step (exon ligation), TES reactions were conducted for one hour in 7 mM $MgCl_2$, 166 nM rP-8/4x ribozyme, 1.33 nM radiolabeled 36-mer substrate, and either 66.5 nM (50X) or 665 nM (500X) 3' exon mimic competitor r(GUGCUCU) (SEQ ID NO:16). The values reported are the average of six independent assays. Likewise, to determine if the 5' product of the first reaction step (5' cleavage) (Figure 1B) is dissociating and then rebinding the ribozyme before the second step

(exon ligation), TES reactions were conducted for one hr in 7 mM $MgCl_2$, 166 nM rP-8/4x ribozyme, 1.33 nM 36-mer non-radiolabeled substrate, and 1.33 nM radiolabeled 5' exon mimic competitor r(AUGACU) (SEQ ID NO:15). In each case the competitors were added simultaneously with the substrates.

Please replace the last paragraph on page 25 and continuing on to page 26 of the specification with the following rewritten paragraph:

Previous studies utilizing the rP-8/4x ribozyme (Testa, S. M., Gryaznov, S. M. & Turner, D. H. (1998) *Biochemistry* **37**, 9379-9385) show that the 5' exon mimic r(AUGACU) (SEQ ID NO:15) binds to the rP-8/4x ribozyme (K_d = 5.2 nM at 37 °C) three orders of magnitude more tightly than the 3' exon mimic r(GUGCUCU) (SEQ ID NO:16) ($K_d \approx$ 20 μM at 37 °C). Interestingly, maximum TES product formation occurs with as little as 20 nM ribozyme (at 44 °C), indicating that for final product formation the 5' and 3' exon intermediates produced during the 5' cleavage step might not dissociate and then rebind the ribozyme before the exon ligation step. To test for 5' exon dissociation and rebinding between the two steps, TES reactions were conducted with 166 nM rP-8/4x, 1.33 nM non-radiolableled 36-mer, and 1.33 nM radiolabeled 5' exon, r(AUGACU) (SEQ ID NO:15). In this case, if the 5' exon intermediate dissociates from the ribozyme, the radiolabeled 5' exon is just as likely to then bind the ribozyme and form the 16-mer product as the non-radiolabeled 5' exon intermediate. As seen in Figure 5, no radiolabeled TES products are observed, indicating the 5' exon intermediate does not dissociate from the ribozyme between the two steps (for those 5' exon intermediates that undergo the complete reaction).

Please replace the last paragraph on page 26 and continuing on to page 27 of the specification with the following rewritten paragraph:

Likewise, to test for 3' exon intermediate dissociation and rebinding between the two reaction steps, TES reactions were conducted with 166 nM rP-8/4x, 1.33 nM radiolabeled 36-mer, and a 50 (66.5 nM) or 500 (665 nM) fold excess of a non-radiolabeled 3' exon mimic competitor, r(GUGCUCU) (SEQ ID NO:16), which would form a 10-mer competition product. At equal molar concentrations if the 3' exon intermediate dissociates from the ribozyme, the 7-mer competitor is 2.5 times more likely to bind the ribozyme and be a substrate in the second reaction step than the 30-mer 3' exon intermediate (data not shown). The results (Figure 5) show

that a 500-fold excess of cold competitor over substrate does not significantly reduce the amount of 16-mer product formed (19.4% ± 2.3% versus 22.8% ± 3%, respectively). The small amount of 10-mer product that is observed at 500-fold excess competitor over substrate (but not 50-fold excess) is not actually competing with the TES reaction. In these cases, the ribozymes that have bound radiolabeled 5' exon regions, and for which the 3' exon region has dissociated, are binding and reacting with a small amount of the huge excess of 3' exon competitor. Therefore, the vast majority of substrates that undergo the complete TES reaction do not have 3' exon intermediate dissociation and rebinding occurring between the two steps of the reaction. Apparently, substrates that undergo only the first reaction step do so because of nearly irreversible 5' or 3' exon intermediate dissociation. It follows that since intermediates to the complete TES reaction do not dissociate from the ribozyme, the TES reaction is intramolecular with regard to substrate.

Please replace the last paragraph on page 27 and continuing on to page 28 of the specification with the following rewritten paragraph:

Excision of a single nucleotide using the *P. carinii.* ribozyme. In order to determine if a lower limit exists to the length of the excised region, we tested the TES reaction using the rP-8/4x ribozyme with two new substrates. One substrate is a 12-mer, r(AUGACUGUGCUC) (SEQ ID NO:~~NO.~~ 7), and was designed to contain the minimum length bridging sequence that could utilize the 2 base pair RE2 interaction (to form the P9.0 helix) and the 3' guanosine thought to be required for self-splicing (Figure 2Bi). The other substrate is a 10-mer, r(AUGACUGCUC) (SEQ ID NO:~~NO.~~ 8), which can not utilize the RE2 interaction, and from which only one nucleotide would be excised (Figure 2Bii). The results (Figure 3A) show that, under the optimal conditions of 10 mM $MgCl_2$ and 44 °C, both the 12-mer and 10-mer reactions lead to the formation of the expected 9-mer products, as confirmed by enzymatic sequencing (data not shown). The optimized reactions produce 72% ± 3.9% product for the 12-mer substrate and 69.3% ± 4.4% product for the 10-mer substrate (for 6 independently run assays). Thus, the rP-8/4x ribozyme can excise as little as a single nucleotide. The same approximate yield is obtained using the 12-mer and 10-mer substrates which suggests that the role of forming the P9.0 helix is not large in this case. Therefore, the RE2 interaction, although perhaps beneficial, is not required for sequence specific TES reactions. In addition, the 12-mer and 10-mer substrates lead

to more than twice the product as compared with the 36-mer substrate, implicating the longer bridging region (which includes the four 5' bases of the intron) as being detrimental for this reaction. As the amount of substrate that undergoes at least the first reaction step is similar for all of the different substrates, 3' exon intermediate dissociation for the 36-mer likely accounts for the difference in extent of final product formation. The dependence of the 10-mer substrate reaction on $MgCl_2$ concentration, time, and rP-8/4x concentration is shown in Figure 3C. The k_{obs} for the first and second step of the reaction are 4.12 and 2.89 min^{-1}, respectively. In contrast to that for the 36-mer, the reaction with the 10-mer substrate is more favorable at $MgCl_2$ concentrations greater than 7 mM, and the second reaction step occurs approximately 50-fold faster. The origin of this effect is unknown, but could be due to the reduced steric hindrance of the smaller bridge on the required conformational rearrangement between the two reaction steps. This could reflect an increased affinity or accessibility of the 3' guanosine of the bridge for the G-binding site of the ribozyme. Indeed, previous reports suggest that the ability of the G-binding site to bind this endogenous guanosine drives the second step of the reaction (Mahadevan, M., Tsilfidis, C., Sabourin, L., Shutler, G., Amemiya, C., Jansen, G., Neville, C., Narang, M., Barcelo, J., O'Hoy, K., et al (1992) *Science* **255**, 1253-1255; Harley, H.G., Rundle, S.A., MacMillan, J.C., Myring, J., Brook, J.D., Crow, S., Reardon, W., Fenton, I., Shaw, D.J., & Harper, P.S. (1993) *Am. J. Hum. Genet.* **52**, 1164-1174).

Please insert after page 30, but before the claims, the attached paper substitute Sequence Listing in the specification.

Attachments: Substitute Sequence Listing (paper copy)
Substitute Sequence Listing (computer readable disk copy)